REMARKS

Claims 1-8, 15-20 and 55-86 were pending in the present application prior to the instant Amendment, of which claims 1-8, 15-20 and 55-72 were allowed, and claims 73-86 were rejected. With the instant Amendment, claims 73, 85 and 86 are amended. Upon entry of this Amendment, claims 1-8, 15-20 and 55-88 will be pending and claims 73-86 will be under consideration.

I. Amendments to the Claims

Claim 73 has been amended to recite in part "A method for modulating conditions associated with a metabolic disorder in a host ... wherein said metabolic disorder is selected from the group consisting of non-insulin-dependent diabetus mellitus (NIDDM), obesity and hypercholesterolemia." Support for this amendment may be found in the specification, for example, at page 9, lines 20-34, at page 22, lines 26-30, and at page 27, lines 11-14 of the application as filed, where the metabolic disorders NIDDM, obesity and hypercholesterolemia are positively recited.

Claim 85 has been amended to recite "The method of claim 73, wherein said metabolic disorder is NIDDM."

Claim 86 has been amended to depend from claim 73.

The amendments to the claims are fully supported by the application as filed and introduce no new matter.

No claim amendment fee is believed to be due.

Applicants respectfully submit that the amendments to claims 73, 85 and 86 should require only a "cursory review" and are in condition for allowance pursuant to M.P.E.P. § 714.13.

Applicants therefore respectfully request entry of these amendments pursuant to 37 C.F.R. § 1.116(b)(1).

II. Interview Summary

A telephone Interview was held on June 4, 2009 between Applicants' representative David C. Pauling ("Applicants' attorney"), and Examiner Margaret D. Seaman ("the Examiner"). Applicants and Applicants' attorney thank the Examiner for the courtesies extended during the Interview. In the Interview, Applicants' attorney and the Examiner

discussed the rejections of claims 73-86 under 35 U.S.C. § 112, first paragraph, for alleged lack of written description and enablement, raised in the Final Office Action mailed March 11, 2009 ("Office Action"). The Examiner indicated that she would favorably consider an amended claim 73 which recites the metabolic disorders "non-insulin-dependent diabetus mellitus, obesity and hypercholesterolemia," if Applicants could provide evidence of a nexus between the modulation of the PPARγ receptor and treatment of these metabolic disorders. Applicants' attorney agreed to amend claim 73 as suggested by the Examiner, and indicated that he would provide a reference providing such evidence. The Examiner indicated that she would cite the reference in a PTO-892 form ("Notice of References Cited").

III. Rejection of Claims 73-86 under 35 U.S.C. § 112, First Paragraph: Written Description Requirement

Claims 73-86 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants respectfully traverse the rejection.

A. The legal standard for written description

To comply with 35 U.S.C. § 112, an application must, *inter alia*, provide a written description of the claimed invention demonstrating the inventors' possession of the invention as claimed. *See* 35 U.S.C. § 112, first paragraph and *New Railhead Mfg., L.L.C. v. Vermeer Mfg. Co.*, 63 USPQ2d 1843, 1846 (Fed. Cir. 2002).

Applicants submit that the specification provides sufficient disclosure of a method for modulating conditions associated with a metabolic disorder, wherein the metabolic disorder is NIDDM, obesity or hypercholesterolemia, such that a person skilled in the art would recognize that the inventors had possession of the recited subject matter of amended claim 73, and claims 74-86 which depend therefrom.

B. The specification demonstrates possession of the claimed subject matter

According to the Examiner:

> It is not seen where the instant specification adequately describes the nexus between the modulation of the PPARγ receptor and a useful treatment of a single disease or condition. Further, it is not seen where the instant specification provides written description [of] diseases and conditions that are associated with metabolic disorders or inflammatory disorders.

See Office Action, section 2, page 3.

Claim 73 as amended recites in part "A method for modulating conditions associated with a metabolic disorder in a host ... wherein said metabolic disorder is selected from the group consisting of non-insulin-dependent diabetus mellitus (NIDDM), obesity and hypercholesterolemia."

At the outset, Applicants respectfully submit that since amended claim 73 recites a method for modulating conditions associated with metabolic disorders only, the allegation made by the Examiner respecting diseases and conditions associated with inflammatory disorders is moot.

With regard to the allegation made by the Examiner respecting diseases and conditions associated with metabolic disorders, Applicants submit that the specification demonstrates Applicants' possession of a method for modulating a metabolic disorder wherein the metabolic disorder is NIDDM, obesity or hypercholesterolemia at, for example, page 9, lines 20-28 and 32-34, page 22, lines 26-29, and page 27, lines 11-14, of the application as filed. Page 9, lines 20-28, discloses that "By activating the PPARγ receptor, the compounds will find use as therapeutic agents capable of modulating conditions mediated by the PPARγ receptor. [An] example of such conditions is NIDDM." Page 9, lines 32-34, discloses that "Compounds that act via antagonism of PPARγ are useful for treating obesity, hypertension, hyperlipidemia, hypercholesterolemia, hyperlipoproteinemia, and metabolic disorders. Page 22, lines 26-30 discloses that "the present invention provides methods for modulating conditions mediated by PPARγ in a host. More particularly, the conditions are selected from non-insulin-dependent diabetes mellitus, obesity, [and] conditions associated with abnormal plasma levels of lipoproteins or triglycerides." Page 27, lines 11-14, discloses initial dosages and dosage ranges for administration of the compounds for therapeutic use in the treatment of obesity and NIDDM.

Further, the specification teaches a nexus between modulation of the PPARγ receptor and a useful treatment of conditions associated with a metabolic disorder:

> PPARγ plays a pivotal role in the adipogenic signaling cascade. PPARγ also regulates the ob/leptin gene which is involved in regulating energy homeostasis, and adipocyte differentiation which has been shown to be a critical step to be targeted for anti-obesity and diabetic conditions.

See application as filed, page 2, lines 4-8.

As such, Applicants respectfully submit that one of skill in the art, reading the instant specification would reasonably conclude that the inventor had possession of a method for

modulating conditions associated with a metabolic disorder wherein the metabolic disorder is NIDDM, obesity or hypercholesterolemia.

Although the amendment to claim 73 and the disclosures in the specification should sufficiently address the Examiner's rejection under 35 U.S.C. § 112, first paragraph, written description requirement, Applicants wish to further explain the state of the art relating to the PPARγ agonism, for purposes of clarifying the nexus between the modulation of the PPARγ receptor and a useful treatment of diseases and conditions associated with metabolic disorders.

Applicant submit herewith a reference as **Exhibit A** by I. W. Campbell, *Current Molecular Medicine*, 2005, 5:349-363, entitled "The Clinical Significance of PPAR Gamma Agonism" (hereinafter "Campbell"). Figure 1 of Campbell describes the inter-relationship between insulin resistance, central obesity and dyslipidaemia (increased triglycerides, decreased HDL-cholesterol, and increased low density LDL particles, indicators of a hypercholesterolemic state). Figure 2 of Campbell describes the mechanism of action of a PPARγ agonist, thiazolidinedione (TZD), on glucose and fatty acid metabolism. Campbell provides the following explanation of Figure 2 on page 351, third paragraph:

> Located in the nucleus, the PPARγ binding site is a heterodimer that becomes an active transcription factor when PPARγ agonists bind to the receptor Fig (2). The transcription factor binds to the regulating site of the specific genes and those genes activated include lipoprotein lipase, fatty acid transporter protein, Acyl CoA synthetase and glucose-4 transporter (GLUT4). Fatty acid and glucose uptake are increased. In addition, TZDs promote adipogenesis through their PPARγ activity stimulating the differentiation of stem cells into adipocytes. Clinically, the TZDs are insulin sensitizers potentiating the action of insulin on adipose tissue with increased lipogenesis. The lowering of serum free fatty acids increases insulin sensitivity in the liver, with decreased hepatic glucose output, and in the muscle wall with increased glucose uptake.

See Campbell, page 351. Campbell further describes the clinical use of thiazolidinediones to treat central obesity, glucose intolerance and type 2 diabetes, and dyslipidaemia.

C. The rejection should be withdrawn

In view of the foregoing, Applicants respectfully request withdrawal of the rejection of claims 73-86 for lack of written description under 35 U.S.C. § 112, first paragraph.

IV. Rejection of Claims 73-86 under 35 U.S.C. § 112, First Paragraph: Enablement Requirement

Claims 73-86 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants respectfully traverse the rejection.

A. The legal standard for enablement

To comply with 35 U.S.C. § 112, an application must, *inter alia*, enable one of skill in the art to make and use the claimed invention. *See* 35 U.S.C. § 112, first paragraph and *New Railhead Mfg., L.L.C. v. Vermeer Mfg. Co.*, 63 USPQ2d 1843, 1846 (Fed. Cir. 2002).

The test is whether one reasonably skilled in the art could make or use the invention, without undue experimentation, from the disclosure in the patent specification coupled with information known in the art at the time the patent application was filed. *U.S. v. Telectronics Inc.*, 857 F.2d 778, 8 USPQ2d 1217 (Fed. Cir. 1988). Undue experimentation is experimentation that would require a level of ingenuity beyond what is expected from one of ordinary skill in the field. *Fields v. Conover,* 170 USPQ 276, 279 (CCPA 1971). The factors that can be considered in determining whether an amount of experimentation is undue have been summarized in *In re Wands,* 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Among these factors are: the amount of effort involved, the guidance provided by the specification, the presence of working examples, the amount of pertinent literature and the level of skill in the art (the "*Wands* factors"). *Id.* While the presence of working examples is to be considered in determining whether the amount of experimentation is undue, "quantity of examples is only one factor which must be considered before reaching a final conclusion" regarding this issue. MPEP §2164.06. The test for undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible, so long as it is merely routine. *In re Wands*, 8 USPQ2d at 1404.

The Examiner recites the *Wands* factors and concludes that undue experimentation is required to practice the rejected claims. Applicants respectfully submit that an analysis of the *Wands* factors requires the opposite conclusion for claims 73-86, and in view of the sufficient nexus known in the art between the modulation of the PPARγ receptor and treatment of diseases and conditions associated with metabolic disorders.

B. The art demonstrates a sufficient nexus between the modulation of the PPARγ receptor and treatment of metabolic disorders to enable to claims

The Examiner alleges that the specification does not enable a correlation between the modulation of PPARγ receptors and the modulation of conditions associated with metabolic or inflammatory disorders. *See*, for example, Office Action, section 3, page 6, lines 19-21 ("The amount of direction or guidance present").

As explained above, amended claim 73 recites in part "A method for modulating conditions associated with a metabolic disorder in a host ... wherein said metabolic disorder is selected from the group consisting of non-insulin-dependent diabetus mellitus (NIDDM), obesity and hypercholesterolemia."

At the outset, Applicants respectfully submit that since amended claim 73 recites a method for modulating conditions associated with metabolic disorders only, the allegation made by the Examiner respecting diseases and conditions associated with inflammatory disorders is moot.

With regard to the allegation made by the Examiner respecting diseases and conditions associated with metabolic disorders, Applicants submit that a sufficient nexus is demonstrated in the art between the modulation of the PPARγ receptor and treatment of diseases and conditions associated with metabolic disorders to enable a correlation between the two. For example, as explained above, Campbell (*Current Molecular Medicine*, 2005, 5:349-363) describes the mechanism of action of a PPARγ agonist, thiazolidinedione, on glucose and fatty acid metabolism, and further describes the clinical use of thiazolidinediones to treat central obesity, glucose intolerance and type 2 diabetes, and dyslipidaemia. Accordingly, one of skill in the art would appreciate that a method for a modulating a PPARγ receptor comprising administration of one of the claimed compounds could achieve its intended result of modulating a condition associated with a metabolic disorder, for example, NIDDM, obesity or hypercholesterolemia, as recited in amended claim 73, and claims 74-86 which depend therefrom.

C. Wands analysis

To the extent that the Examiner considers that the *Wands* factors support a finding of undue experimentation for the subject matter of claims 73-86, Applicants present the following additional arguments.

1. The state of the prior art and the predictability in the art

> According to the Examiner:
>
> The state of the prior art is that it involves screening in vitro and in vivo to determine which compounds exhibit the desired pharmacological activities.... There is no absolute predictability in view of the seemingly high level of skill in the art. The existence of these obstacles establishes that the contemporary knowledge in the art would prevent one of ordinary skill in the art from accepting any therapeutic regimen on its face.

See Office Action, section 3, page 4 ("The state of the prior art").

> Further, according to the Examiner:
>
> [I]n the absence of a showing of a nexus between any and all known diseases associated with metabolic or inflammatory disorders and the modulation of PPARγ receptors, one of ordinary skill in the art is unable to fully predict possible results from the administration of the compound of claim 1 due to the unpredictability of the role of modulation of PPARγ receptors.

See Office Action, section 3, pages 4-5 ("The predictability in the art").

As explained above, the art demonstrates a nexus between the modulation of the PPARγ receptor and a useful treatment of diseases and conditions associated with metabolic disorders, as disclosed in, for example, Campbell. Accordingly, one of skill in the art would have appreciated that a compound capable of modulating a PPARγ receptor might also be useful in a therapeutic regimen for treating such metabolic disorders as NIDDM, obesity and hypercholesterolemia.

2. The presence or absence of working examples

> According to the Examiner:
>
> Several compounds have been shown to lower glucose levels.... However, this has not been linked with the modulation of conditions associated with metabolic or inflammatory disorders. Nor have any specific conditions associated with metabolic or inflammatory disorders been assayed or treated with the instant compounds.

See Office Action, section 3, page 6 ("The presence or absence of working examples").

As conceded by the Examiner, the specification teaches that the claimed compounds lower glucose levels (*see*, for example, Example 374, pages 198-199). Further, as explained above, Campbell describes the clinical use of thiazolidinediones for the treatment of central obesity, glucose intolerance and type 2 diabetes, and dyslipidaemia. Moreover, Applicants respectfully remind the Examiner that it is not necessary that a patent applicant test all the embodiments of his invention. *See Amgen Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d

1200, 1213, 18 USPQ 2d 1016 (Fed. Cir. 1991). 35 U.S.C. § 112 requires simply that the patent applicant provide a disclosure which sufficiently enables one of skill in the art to carry out the invention commensurate with the scope of the claims. *Amgen*, 927 F.2d at 1213.

3. The amount of direction or guidance present

According to the Examiner:

> The specification does not seem to enable a correlation between the mediation of PPARγ receptors and the modulation of conditions associated with metabolic or inflammatory disorders.

See Office Action, section 3, page 6 ("The amount of direction or guidance present").

The specification teaches how to use a method for modulating NIDDM, obesity and hypercholesterolemia at, for example, pages 24-25 ("Analysis of the Compounds"), page 27, lines 11-14, and Examples 373, pages 197-198, of the application as filed. Pages 24-25 and Example 373 teach assays for evaluation of the claimed compounds for modulation of a PPARγ receptor. Page 27, lines 11-14, teaches initial dosages and dosage ranges for administration of the claimed compounds for therapeutic use in the treatment of obesity and NIDDM. Further, as discussed above, the specification teaches a nexus between modulation of the PPARγ receptor and a useful treatment of conditions associated with a metabolic disorder at, for example, page 2, lines 4-8 of the application as filed.

Applicants submit that based on the teachings of the specification, one of skill in the art, using no more than standard assaying techniques and routine experimentation, could determine which of the claimed compounds are active as PPARγ receptor modulators, and could further administer such compounds to a host at an appropriate dosage level, with an expectation that such administration could be useful in the treatment of conditions associated with a metabolic disorder.

4. The quantity of experimentation needed

According to the Examiner:

> The quantity of experimentation needed is undue. One skilled in the art would need to determine what diseases out of all known diseases associated with metabolic or inflammatory disorders would be benefited by the mediation of PPARγ receptors and then would further need to determine which of the claimed compounds would provide treatment of the disease.

See Office Action, section 3, page 7 ("The quantity of experimentation needed").

Applicants submit that based on the teachings of the specification and the nexus demonstrated in the art between the modulation of the PPARγ receptor and a treatment of diseases and conditions associated with metabolic disorders, one of even ordinary skill in the art would be able to use the claimed methods without undue experimentation.

5. *The level of skill in the art*

The Examiner acknowledges that the level of skill in the art is high. This is a factor in Applicants' favor.

D. The claims are enabled

Applicants respectfully submit that the teachings of the specification, the nexus demonstrated in the art between the modulation of the PPARγ receptor and a treatment of diseases and conditions associated with metabolic disorders, and the high level of skill in the art would enable one to use a method recited in amended claim 73, and claims 74-86 which depend therefrom, without undue experimentation.

E. The rejection should be withdrawn

In view of the foregoing, Applicants respectfully request withdrawal of the rejection of claims 73-86 for lack of enablement under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In light of the foregoing amendments and remarks, Applicants respectfully request that the Examiner reconsider this application with a view towards allowance. The Examiner is invited to call the undersigned at (650) 739-3949 if she believes a telephone call could expedite allowance of the instant claims.

No fee is believed due with this response. However, should the Commissioner determine otherwise, the Commissioner is hereby authorized to charge any required fee(s) or credit any overpayment to Jones Day Deposit Account No. 50-3013 (order no. 893053-999123).

Respectfully submitted,

Date: June 11, 2009

David C. Pauling 56,056
For: Anthony Insogna (Reg. No.35,203) (Reg. No.)

JONES DAY
222 East 41st Street
New York, New York 10017
(212) 326-3939

Exhibit A: I. W. Campbell, "The Clinical Significance of PPAR Gamma Agonism," *Current Molecular Medicine*, 2005, 5:349-363

The Clinical Significance of PPAR Gamma Agonism

I.W. Campbell*

Consultant Physician, Victoria Hospital, Kirkcaldy and Honorary Professor, Department of Medical Sciences, University of St Andrews, Scotland

Abstract: Insulin resistance is a principal underlying defect in type 2 DM along with beta-cell dysfunction, and this insulin resistance underpins many of the abnormalities associated with the metabolic syndrome. Peroxisome-proliferator-activated receptor gamma agonists (PPARγ agonists), also known as glitazones or thiazolidinediones (TZDs) are powerful insulin sensitisers with recent evidence suggesting that they also have a potential to improve pancreatic beta-cell function.

TZDs cause a major redistribution of body fat with a decrease in visceral and hepatic fat content with a resultant increase in insulin sensitivity. The glucose lowering effects of TZDs are similar to those seen with the well-established sulphonylureas and metformin. TZDs have a small reducing effect on blood pressure and have been shown to reduce microalbuminuria independent of their blood glucose lowering effect. Both TZDs in clinical practice, pioglitazone and rosiglitazone, reduce small dense LDL-cholesterol and increase HDL-cholesterol levels but pioglitazone would appear to have a more pronounced benefit on these two parameters with a greater reduction in plasma triglycerides. TZDs improved the pro-coagulant state and show benefits in improving endothelial dysfunction and reducing 'non-traditional' inflammatory cytokines and increasing adiponectin levels. The greatest benefit for the TZDs is to directly influence atherogenesis itself and the potential that these so-called pleiotrophic effects of TZDs to reduce cardiovascular events in type 2 DM will be tested when the results of outcome trials are published in the next few years. If the results are positive for the reduction in vascular end-points, then TZDs will represent a major advance in improving the prognosis of type 2 DM subjects with the metabolic syndrome.

INTRODUCTION

The prevalence of type 2 diabetes mellitus (DM) throughout the world is now reaching epidemic proportions. The current global prevalence of type 2 DM is about 150 million with a predicted increase to 215 million by 2010 and 300 million by 2025 [1]. Some countries are expected to experience a disproportionally large increase in the occurrence of type 2 DM, in particular India, Pakistan, Indonesia and Mexico.

Type 2 DM, previously known as non-insulin-dependent diabetes mellitus (NIDDM), accounts for 75-90% of all cases of diabetes depending upon the ethnic background. Type 2 DM has previously been erroneously referred to as 'mild diabetes' because it is often asymptomatic with no classical symptoms of diabetes such as thirst or polyuria. However, type 2 DM is a 'silent killer' where cardiovascular disease is the principal cause of death in over 70% of type 2 DM patients. Accelerating atherosclerosis may be present for some years before the onset of clinical diabetes. The life expectancy of a type 2 DM patient is reduced by 8-10 years in the 40-70 age range [2]. Data from the Copenhagen Heart Study shows that in people with type 2 DM, the risk of having a myocardial infarction or stroke is increased 2-3 fold and the risk of death is 2-fold, independent of other known risk factors for cardiovascular disease [3]. It is now appreciated that type 2 diabetes mellitus is not a distinct clinical entity but rather a complex of risk factors for cardiovascular disease (CVD). This clustering of cardiovascular risk factors is now recognised as a syndrome, which is closely linked with insulin resistance and hyperinsulinaemia.

*Address correspondence to this author at the Consultant Physician, Victoria Hospital, Hayfield Road, Kirkcaldy KY2 5AH, Scotland, Tel: +44 1592 643355 ext 8619; Fax: +44 1593 648049; E-mail: jackie.wallace@faht.scot.nhs.uk

INSULIN RESISTANCEANDTHEMETABOLIC SYNDROME

The clustering of CVD risk factors was first described clearly by Reaven in his 1988 Banting lecture who highlighted central obesity, glucose intolerance, hypertension and dyslipidaemia as the key players in the atherosclerotic risk-factor complex. Reaven coined the term Syndrome X [4], others referred to it as Reaven's syndrome or the 'deadly quartet' [5]. The terminology continued to change in the 1990s. The terms insulin resistance syndrome, the pleurimetabolic or dysmetabolic syndrome were suggested but the *metabolic syndrome* is now the term used most frequently. Further characteristics of the metabolic syndrome have been described since Reaven's original description and perhaps the term 'deadly sextet' might be more appropriate than 'quartet'. Since type 2 DM often gives rise to sudden cardiac death with no preceding cardiac history, the term '*silent sextet*' has been proposed to bring together the clinical features of the metabolic syndrome [6].

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Features of the Silent Sextet

The six principal features of the cardiovascular risk cluster seen with the metabolic syndrome are as follows:

1. Central obesity
2. Glucose intolerance and type 2 diabetes
3. Hypertension
4. Dyslipidaemia (↑ triglycerides, ↓ HDL-cholesterol, ↑ low density LDL particles)
5. Pro-coagulant state
6. Endothelial dysfunction, inflammation and atherosclerosis

Fig. (**1**) illustrates how these abnormalities interlink to result in premature cardiovascular disease.

INSULIN RESISTANCE AND BETA-CELL DYSFUNCTION

The principal underlying defects in type 2 DM are reduced insulin sensitivity (insulin resistance) and impaired beta-cell function. The net effect of insulin resistance is a decreased ability of insulin to suppress hepatic glucose production and to decrease muscle and adipose tissue glucose uptake. Insulin resistance plays a major role in the aetiology of atherosclerosis. The beta-cell abnormalities in type 2 DM include a delayed insulin secretion after nutrient stimulation (loss of first phase insulin secretion), an inability to compensate adequately for insulin resistance, progressive beta-cell loss with duration of type 2 diabetes mellitus and a metabolic inhibition of beta-cell function by chronic hyperglycaemia ('glucose toxicity'). PPAR γ agonism reduces insulin resistance, has the potential to improve beta-cell function and has a strong theoretical basis to delay or reverse atherosclerosis through its influence on the 'silent sextet'

PPAR γ AGONISM: GLITAZONES: THIAZOLIDINEDIONES (TZDS)

Peroxisome-proliferator-activated receptors (PPARs) are nuclear receptors that regulate gene transcription. PPAR γ receptors are most strongly expressed in adipose tissue and in the vascular wall with secondary benefits on insulin sensitivity in skeletal muscle and liver. Prostaglandin metabolites are natural ligands for these receptors. PPAR γ agonists have been introduced into clinical practice in the late 1990s. They are also known as

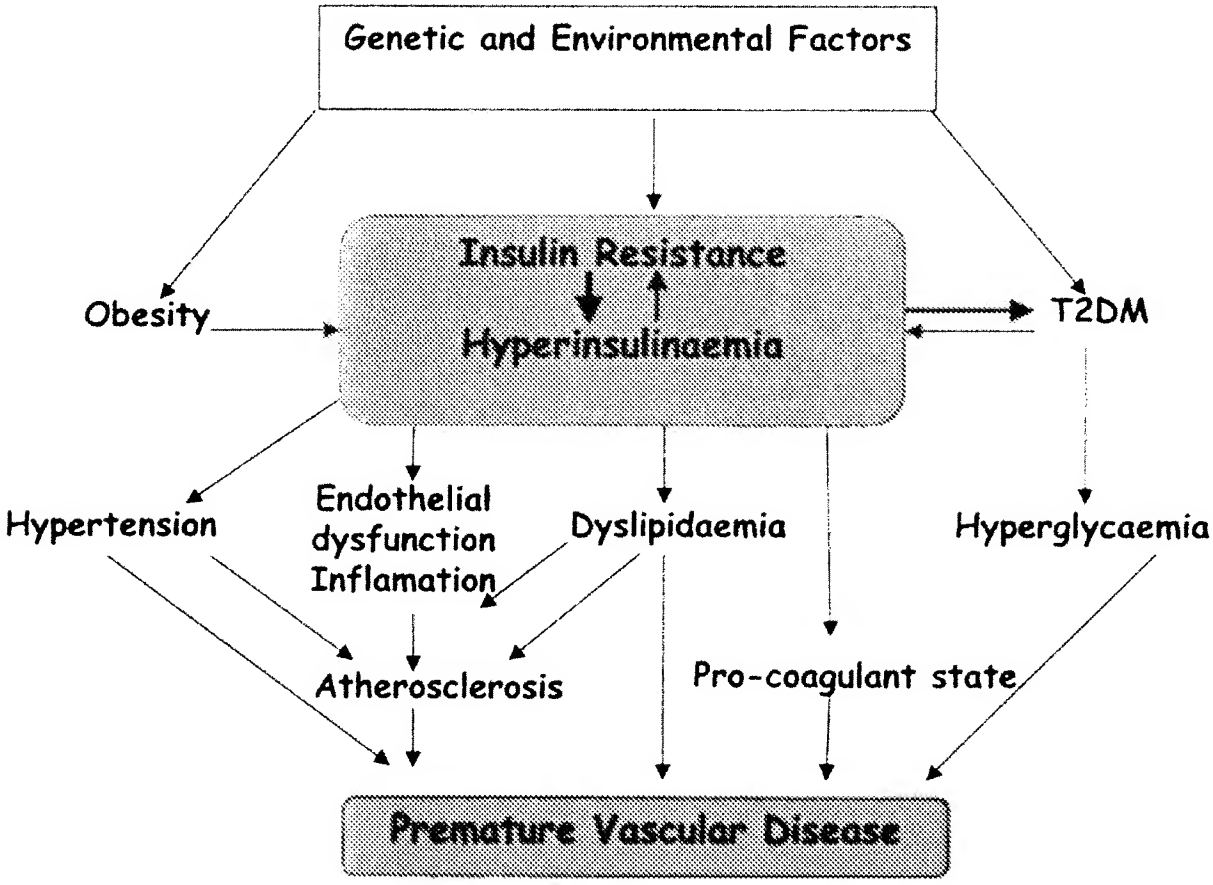


Fig. (1). Inter-relationship of components of metabolic syndrome resulting in increased cardiovascular risk. Adapted from ref 6, with permission.

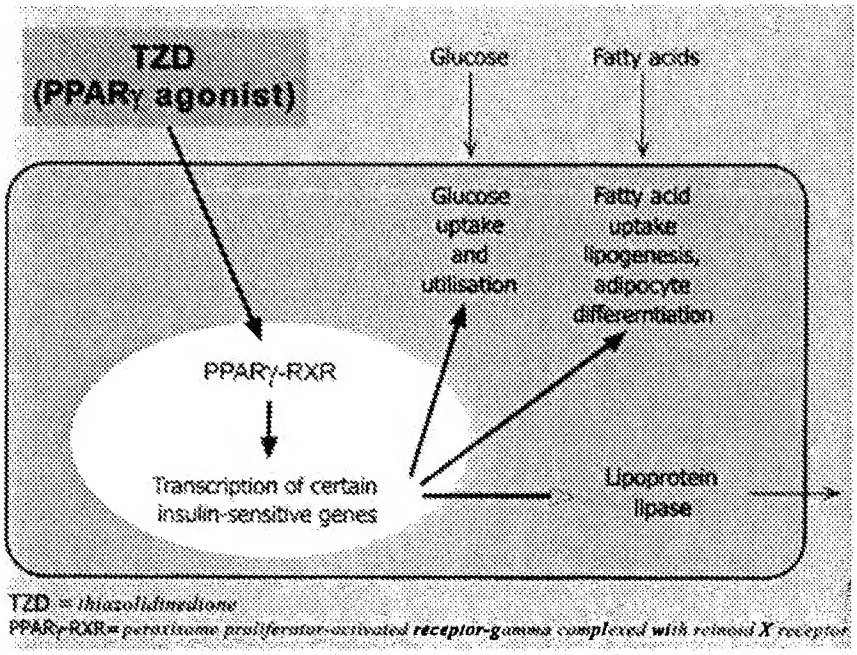


Fig. (2). Mechanism of action of a TZD to active PPARγ receptor (from Bailey CJ, Feher MD, Therapies for diabetes, Sherbourne Gibbs; Birmingham, UK, 2004).

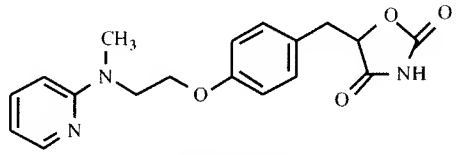
glitazones or TZDs. For the remainder of this review, the term TZDs will be used.

TZDs act at the level of the genome, modifying the transcription of a number of genes that regulate proteins involved in insulin action and lipid metabolism. The TZDs are drug ligands for the PPAR γ receptor.

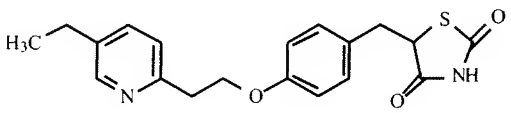
Located in the nucleus, the PPAR γ binding site is a heterodimer that becomes an active transcription factor when PPAR γ agonists bind to the receptor Fig. **(2)**. The transcription factor binds to the regulating site of the specific genes and those genes activated include lipoprotein lipase, fatty acid transporter protein, Acyl CoA synthetase and glucose-4 transporter (GLUT4). Fatty acid and glucose uptake are increased. In addition, TZDs promote adipogenesis through their PPAR γ activity stimulating the differentiation of stem cells into adipocytes. Clinically, the TZDs are insulin sensitizers potentiating the action of insulin on adipose tissue with increased lipogenesis. The lowering of serum free fatty acids increases insulin sensitivity in the liver, with decreased hepatic glucose output, and in the muscle wall with increased glucose uptake.

THIAZOLIDINEDIONES IN CLINICAL PRACTICE

In 1997, the first TZD was introduced into clinical practice. Troglitazone first became available in the USA as an oral hypoglycaemic agent. However it had to be withdrawn in 2000 because of problems of hepatotoxicity (see below). Rosiglitazone and pioglitazone became available for clinical use in 1999 and these are now the two TZDs used world-wide at the present time Fig. **(3)**. How do these new agents affect the 'silent sextet'? Each of the six components of the metabolic syndrome will be reviewed with specific regard as to how the TZDs may provide clinical benefit.



Rosiglitazone



Pioglitazone

Fig. (3). Chemical structure of rosiglitazone and pioglitazone.

EFFECT OF TZD'S ON COMPONENTS OF 'SILENT SEXTET'

Central Obesity

About 70-80% of type 2 DM patients are obese. Central or abdominal obesity, giving an 'apple-

shaped' appearance, underpins the insulin resistance and hyperinsulinaemia, the key central features of type 2 DM Fig. **(4).**

The increased waist-hip (W/H) ratio of > 1.0 in males and > 0.8 in females predicts an increased risk of cardiovascular disease. All cause morbidity and mortality rise as the degree of obesity increases. There is now increasing concern in the rise of childhood obesity and the emergence of type 2 DM in adolescence [7,8]. High caloric intake, limited physical activity and the resultant obesity with insulin resistance are common in populations with a high prevalence of type 2 DM.

All newly diagnosed type 2 DM patients are given appropriate lifestyle advice which includes dietary change to lose weight and encouragement to increase daily exercise activity. Patients are therefore disappointed to be informed that they will most likely gain weight with TZD therapy. Modest weight gain, on average 2-3 kgs in the first 6-12 months of therapy, will occur. Despite this weight gain, patients on TZD treatment have an improved insulin sensitivity and glycaemic control improves. In fact, the greater the weight gain seen in type 2 DM on TZD therapy, the greater is the reduction in the glycated haemoglobin (HbA1c) [9].

TZDs cause a major redistribution of body fat with a decreased in visceral fat, a concomitant increase in subcutaneous fat [10] and a reduction in hepatic fat content [11,12]. Muscle fat levels may also reduce. The mobilisation of fat out of the liver and muscle results in improved hepatic and muscle insulin sensitivity. The reduction in visceral fat in the omentum causes a fall in venous portal free fatty acids (FFAs) transported to the liver and further improved hepatic insulin sensitivity occurs Fig. **(5)**. It is important to explain to the patient that this small weight gain is not detrimental but potentially beneficial in terms of reducing insulin resistance and lessening the risk of atherosclerotic vascular events. It is also important to stress that this

Fig. (4). Typical phenotype of middle-age male with type 2 diabetes and central obesity.

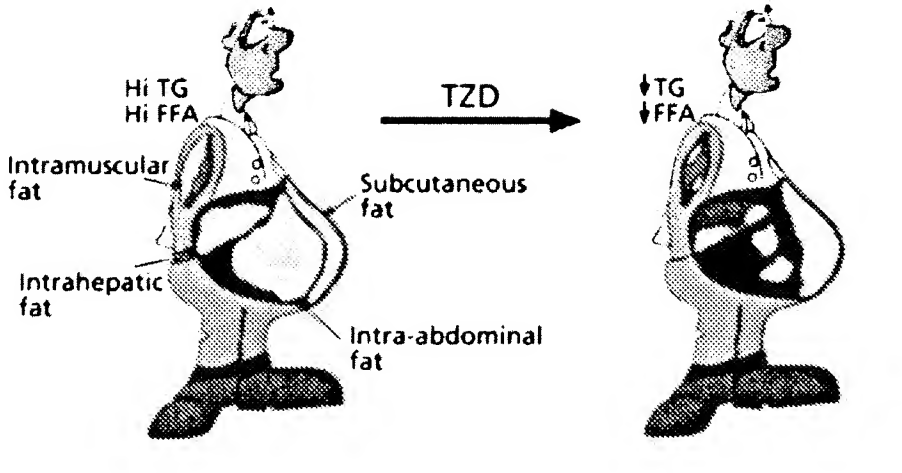


Fig. (5). Effect of TZDs on body fat distribution on type 2 DM patients. Adapted from ref 9, with permission.

weight gain is usually seen in the first six months of TZD therapy and thereafter the weight appears to plateau or even fall a small amount.

GLUCOSE INTOLERANCE AND TYPE 2 DIABETES

Epidemiological data have shown chronic hyperglycaemia is associated with an increased risk of macrovascular disease [13]. The prospective observational data of the UK Prospective Diabetes Study (UKPDS) showed that each 1% reduction in updated mean HbA1c was associated with clinical benefits such as 21% decreased risk of diabetes-related death and a 14% reduction in fatal and non-fatal myocardial infarction [14].

TZDs, rosiglitazone and pioglitazone, lower blood glucose by increasing muscle and adipose tissue glucose uptake and to a lesser degree by decreasing hepatic gluconeogenesis. Adipogenesis, promoted by TZDs, results in an increased uptake of FFAs and glucose, and

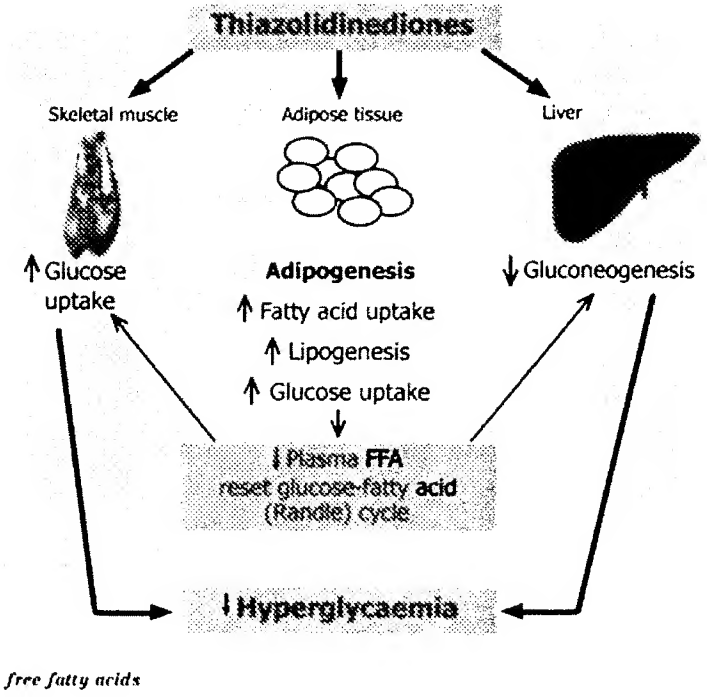


Fig. (6). Mechanism by which TZDs reduce hyperglycaemia (from Bailey CJ, Feher MD, Therapies for diabetes, Sherbourne Gibbs, Birmingham, UK 2004).

decreases the release of FFAs into the circulation. This decreases plasma glucose principally *via* the glucose-fatty acid (Randle) cycle. The reduction in FFA concentration causes a fall in available FFAs in the liver as a source of gluconeogenesis. There is also a reduction in FFAs to muscle tissue which will result in increased glucose uptake Fig. **(6)**.

The many initial short-term trials of 3-6 months duration with both TZDs showed a fall in HbA1c of 0.5-1.5% (on average about 1%), and a reduction of fasting plasma glucose of about 2-3 mmol/L depending upon the circumstances of their use or study [15]. When compared with the long established oral hypoglycaemic agents, the sulphonylureas (e.g. glibenclamide, gliclazide) and the biguanide metformin, both rosiglitazone and pioglitazone have comparable blood glucose lowering efficacy but no greater ability to reduce blood glucose.

The cornerstone of oral hypoglycaemic agent therapy at present is metformin. In the UKPDS, overweight type 2 DM patients treated with metformin benefited from a significant reduction in the incidence of macrovascular end points over the 9-year follow up period: a 42% reduction in diabetes related death and a 39% reduction in myocardial infarction [16]. In terms of glycaemic control TZDs and metformin appear equally effective but patients on metformin tend to lose weight and those on TZDs gain weight. Fig. **(7)** illustrates the similar glycaemic control of pioglitazone versus metformin in diet-failed type 2 DM patients followed up for 32 weeks [17] and sulphonylurea-failure patients treated with either pioglitazone or metformin combination for 52 weeks [18].

In longer-term follow-up studies, four 52-week trials involving 370 patients with type 2 DM, showed that pioglitazone was as effective as metformin or gliclazide as monotherapy and in combination with either of these agents [19]. An interim 18-month analysis showed no difference in HbA1c or fasting plasma glucose between rosiglitazone and metformin or sulphonylurea add-on therapies in sulphonylurea or metformin failure patients where combination therapy was used. Over the 18 month period the change in HbA1c was about 0.5% in all groups [20].

Even longer term data of two years duration with regard to glycaemic control with TZDs has been presented. Pioglitazone showed a better sustainability than gliclazide in reducing fasting plasma glucose although there was no significant differences in HbA1c values (- 0.9% and – 0.7% respectively) [21]. When pioglitazone was compared with gliclazide in combination therapy when either drug was added to metformin, there was again a significant difference in favour of pioglitazone in reduction of fasting plasma glucose but no significant difference in HbA1c levels (- 0.89% and – 0.77% respectively) [22].

There is a trend in the two year data to suggest that the TZD, pioglitazone, may sustain glycaemic control better than the conventional oral hypoglycaemic agents, especially the sulphonylureas. However, about 70% of the pioglitazone patients were titrated to a maximum dose of 45 mg daily whereas only 33% of gliclazide

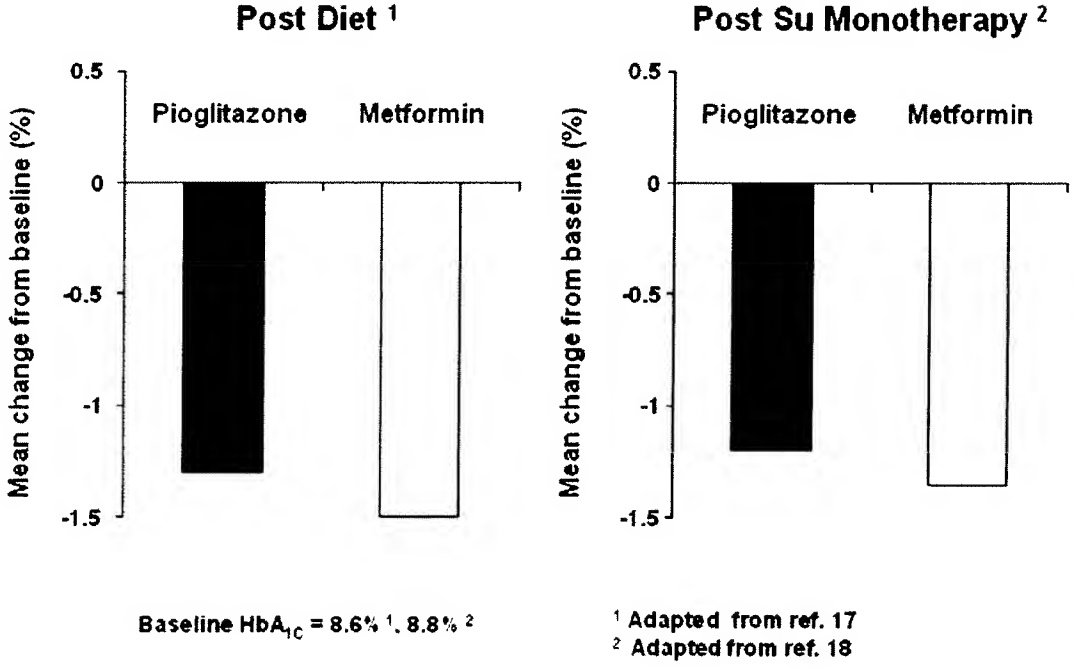


Fig. (7). Pioglitazone versus metformin in diet-failured type 2 DM patients for 32 weeks (ref 17) and sulphonylurea-failure patients treated with either pioglitazone or metformin combination of 52 weeks (ref 18): decrement in HbA1c levels.

patients were titrated to a maximum dose of 320 mg. The preliminary results of these studies require further evaluation by the peer-review process.

The UKPDS has demonstrated the limitations of long-term monotherapy with only 50% of patients able to reach a target HbA1c of 7% after 3 years [23]. Most type 2 DM patients will require combination therapy, with at least two differently acting agents [24]. The introduction of TZDs has increased the range of drug combinations available. With insulin resistance being such an important feature of type 2 DM, the combination of metformin with a TZD will unite two classes of agents with complimentary effects in reducing blood glucose. TZDs act principally to stimulate glucose uptake and utilisation by muscle whereas metformin suppresses hepatic glucose production more strongly than it stimulates muscle glucose uptake. Such a combination of rosiglitazone-metformin is now available for clinical use ('Avandamet') [25]. A similar combination agent incorporating metformin and pioglitazone ('Actoplus Met') has been submitted for approval in USA in October 2004.

HYPERTENSION

Approximately 40% of type 2 DM patients will be hypertensive by the age of 40-50 years, this figure rising to approximately 60% by 70 years. The co-existence of high blood pressure and diabetes is associated with a doubling of the presence of microalbuminuria, left ventricular hypertrophy, ECG abnormalities e.g. of previous myocardial infarction, and a previous history of overt cardiovascular events. Microalbuminuria is closely linked with hypertension in type 2 DM and is a recognised marker of accelerated vascular disease and premature death [26]. Tight blood pressure control substantially reduces the risk of microvascular complications. With regard to macrovascular complications, the UKPDS prospective observational study showed that a 10 mmHg reduction in systolic blood pressure will reduce the risk of diabetes-related death, fatal and non-fatal myocardial infarction, stroke and heart failure by 17%, 12%, 19% and 12% respectively [27].

Hypertension is linked with insulin resistance and therefore it would be expected that TZDs which reduce insulin resistance might also have beneficial effects on blood pressure in type 2 DM patients. This is the case but the reductions are small and not consistent in all the trials. On average, these reduction have ranged from 3 mmHg to 9 mmHg in mean arterial pressure [28].

In a study of 203 type 2 DM patients, rosiglitazone 4 mg bd given as monotherapy significantly reduced the 24-hour ambulatory systolic blood pressure from baseline by 3.5 mmHg when compared with the sulphonylurea, glibenclamide, 10.5 mg daily with a significant reduced diastolic blood pressure of 2.7 mmHg and a mean arterial pressure of 2.8 mmHg Fig. **(8)** [29].

In a larger number of 759 type 2 DM patients in the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of glycaemia in Diabetes

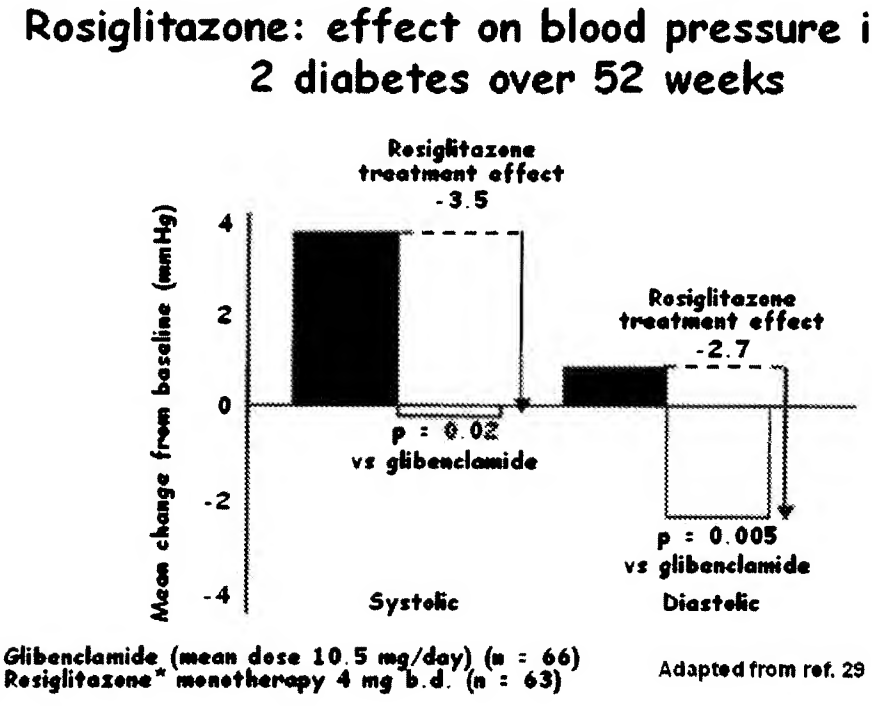


Fig. (8). Effect of rosiglitazone on blood pressure in type 2 diabetes over a 52 week period.

(RECORD) study, those patients inadequately controlled on metformin were randomised to add-on therapy with rosiglitazone or a sulphonylurea or those inadequately controlled with a sulphonylurea were randomly assigned to add-on treatment with rosiglitazone or metformin. At baseline 24 h mean systolic and diastolic blood pressures were comparable between treatment groups. At 6 months, adding rosiglitazone to sulphonylurea therapy, resulted in statistically significant reduction in ambulatory blood pressure when compared with sulphonylurea plus metformin combination with approximately 3 mmHg reductions difference in systolic and diastolic pressures [30].

Therefore the effect of TZDs on blood pressure is a small one but in the right direction but will not be of a magnitude to negate treatment of hypertension in type 2 DM patients with renin-angiotensin blocking agents or other drugs. Indeed to achieve the target level of control of blood pressure in type 2 DM of 140/80 mmHg or less, most patients with type 2 DM and hypertension will require two or more anti-hypertensive agents. In the UKPDS, about 30% of the 'tight control' group required three or more different drugs at 6 years of follow-up [31]. In many cases drugs which block the renin-angiotensin system, ACE inhibitors or angiotensin type 1 receptor blockers (ARBs) are often drugs of choice in treating hypertension in type 2 DM. Recent experimental data has shown that a specific subset of ARBs, irbesartan and telmisartan, induces PPAR γ activity, providing a potential mechanism for the pleiotrophic and anti-diabetic effects of ARBs independent of their angiotensin receptor blocking action [32].

TZDs have been shown to reduce microalbuminuria. In a 52 week study, rosiglitazone significantly reduced the albumin-creatinine ratio (ACR) from baseline by 26% in all 121 patients and by 54% in those patients with microalbuminuria at baseline Fig. **(9)**. In contrast the sulphonylurea treatment with glibenclamide did not show any significant reduction in the ACR over the same period [33]. In those patients with microalbuminuria at baseline, the changes in ACR correlated significantly with changes in mean systolic and diastolic blood pressure in the rosiglitazone treated group. As the degree of glycaemic control was similar in both the rosiglitazone and glibenclamide groups, the effect of rosiglitazone on urinary albumin excretion is independent of its metabolic effects. It may be the PPAR γ agonist gives vascular and endothelial protection.

Pioglitazone's effect on urinary albumin excretion has also been shown over a one year period in combination therapy. Urinary ACR was reduced by 15% in the pioglitazone and sulphonylurea combination group and increased by 2% in the sulphonylurea plus metformin group [18]. Again this difference was independent of blood glucose and was not related to changes in blood pressure or use of renin-angiotensin blocking drugs (about 44% in both groups). This degree of

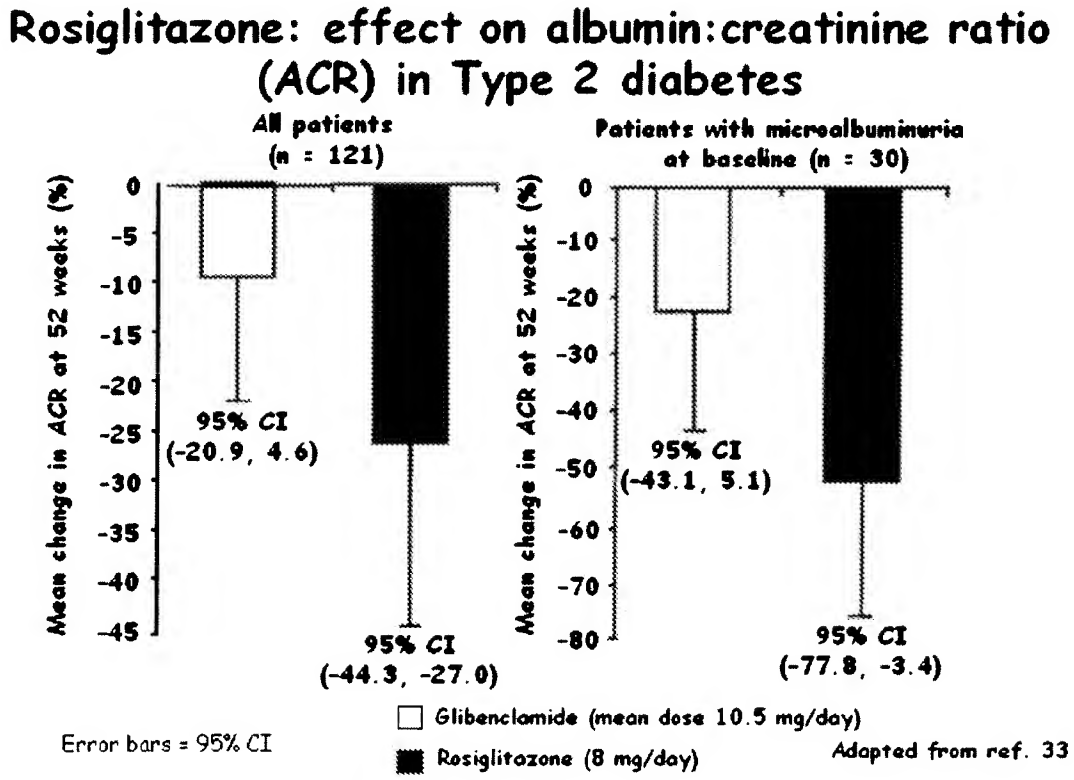


Fig. (9). Effect of rosiglitazone compared to glibenclamide on albumin: creatinine ratio (ACR) in type 2 diabetes.

improvement in the cardiovascular risk marker, microalbuminuria, is of similar order to that seen with angiotensin converting enzyme (ACE) inhibitors. This effect may prove to be of value in high risk type 2 DM patients.

DYSLIPIDAEMIA

Many patients with type 2 DM display an atherogenic lipoprotein phenotype of moderately elevated triglyceride levels, elevated small dense LDL and decreased HDL, especially HDL_2, cholesterol levels. In the UKPDS, a study of patients who developed coronary artery disease identified five potentially modifying risk factors to have strong predictive value at diagnosis of patients who would develop heart disease. Ranked in order of prognostic importance, these were dyslipidaemia (increased LDL-cholesterol, decreased HDL-cholesterol), increased levels of glycated haemoglobin, hypertension and cigarette smoking [34]. In addition hypertriglyceridaemia has also been shown to be an independent risk factor for coronary artery disease [35].

Although subjects with type 2 DM and dyslipidaemia should be treated with lipid-lowering agents such as statins, fibrates and nicotinic acid, the TZDs may provide additional benefits in combination with these agents. In clinical practice there is continuing debate concerning the potential differences between rosiglitazone and pioglitazone on plasma lipids.

Rosiglitazone and pioglitazone were compared in an open-label trial in 127 type 2 DM patients previously treated with troglitazone. When the latter TZD was withdrawn, there was a 2-week wash-out period and patients randomly assigned to either rosiglitazone or pioglitazone and lipid profiles were assessed at randomisation and at 4 months follow-up. No significant difference in lipid profile was seen with rosiglitazone but significant improvements were seen with pioglitazone in regard to LDL cholesterol and triglyceride reductions [36]. In a 12-month multi-centre, double-blind, randomised control parallel-group trial of type 2 DM patients with features of the metabolic syndrome, and who did not respond satisfactorily or had adverse reactions with sulphonylurea or metformin, the combination of glimepiride plus pioglitazone was associated with a significant improvement in lipid and lipoprotein variables, whereas the combination of glimepiride plus rosiglitazone had no such benefits on lipid metabolism. In particular, the pioglitazone showed significant decrease in triglyceride levels and a significant increase in HDL-cholesterol levels [37].

Van Wijk *et al.* performed a retrospective analysis of 19 published double-blind placebo controlled studies with rosiglitazone and pioglitazone, involving 5304 patients, 3236 patients in studies involving rosiglitazone and 2068 in studies where pioglitazone was used [38]. Studies with pioglitazone showed greater reductions on triglycerides, total cholesterol and LDL cholesterol after adjustment for respective lipid levels at baseline. However these subjects treated with pioglitazone were more obese, had poorer glycaemic control and more pronounced dyslipidaemia with increased triglycerides and decreased HDL-cholesterol levels. These differences in study population characteristics may have influenced the difference in lipid results between the two TZDs.

In a more recent analysis of 11 studies looking at the effect of TZDs on serum lipids and lipoproteins, both pioglitazone and rosiglitazone increased HDL-cholesterol levels by about 10%, rosiglitazone but not pioglitazone increased LDL-cholesterol levels by 8-16%, and pioglitazone had greater reductions in triglyceride levels of about 20% on average [39].

Following the publication of these retrospective analyses, there is now long term follow up data showing the effect of TZDs on serum lipids. At the end of a two year period, pioglitazone had superior effects on triglycerides, HDL-cholesterol and atherogenic index of plasma (AiP), which is inversely correlated with LDL particle size, as add-on therapy compared with gliclazide or metformin. Pioglitazone plus metformin compared with gliclazide and metformin therapy showed a 23% difference in rise of HDL-cholesterol from baseline over the two year period ($p < 0.001$) and the combination of pioglitazone plus gliclazide showed a 21% difference in rise of HDL-cholesterol compared to metformin and gliclazide combination. The beneficial effects of pioglitazone and triglycerides were confirmed, the LDL-cholesterol levels were not modified from baseline at the end of two years and there was a reduction in dense LDL-particles compared to the comparator drugs gliclazide and metformin [40].

The trials mentioned, either singly or as part of literature reviews, comparing rosiglitazone and pioglitazone were either retrospective or clinical trials not rigorously controlled for concomitant glucose and lipid lowering therapies. A multi-centre, prospective, randomised, double-blind, comparative study of these TZDs in patients with type 2 DM and dyslipidaemia, receiving no other concomitant glucose or lipid lowering therapies, was presented at the American Heart Association meeting in November 2004. Table **1** shows the 24 week changes from baseline in this head-to-head comparison of the two TZDs. The study demonstrated that pioglitazone and rosiglitazone exert different effects on plasma lipids. Pioglitazone treatment was associated with significant improvements versus rosiglitazone on triglycerides, HDL-cholesterol, non-HDL-cholesterol, LDL particle size and concentration, despite similar effects on glycaemic control [41]. A suggested molecular mechanism for these differences is that pioglita-

Table 1. Changes in Lipid Parameters from Baseline to Week 24 in Type 2 DM Patients Treated with Pioglitazone or Rosiglitazone

Measure	PIOGLITAZONE	(n = 363)	ROSIGLITAZONE	(n = 356)
	Mean change	% change	Mean change	% change
	± SEM		± SEM	
• Triglycerides (mg/dl)	-51.9 ± 7.8 *	-12.0 *	+13.1 ± 7.8	+14.9
• HDL-C (mg/dl)	+5.2 ± 0.5 *	+14.9 *	+2.4 ± 0.5	+7.8
• LD-C (mg/dl)	+12.3 ± 1.6 *	+15.7 *	+21.3 ± 1.6	+23.3
• LDL particle concentration	-50.5 ± 21.7 *	-7.8 *	+110.5 ± 21.5	+12.0

* $p < 0.001$ between treatment groups

zone may have PPARα activity compared with rosiglitazone. Whether these differences in lipid parameters translate into increased benefits for pioglitazone in reducing cardiovascular complications remains to be determined.

PRO-COAGULANT STATE

Type 2 DM is associated with several defects in the pathways of coagulation and fibrinolysis. These features have implications for both microvascular and macrovascular complications of diabetes. Elevated fibrinogen concentrations and increased blood viscosity are associated with insulin resistance and hyperinsulinaemia, as is the increase in Von Willebrand factor which induces platelet activation and aggregation. Plasminogen activator inhibitor-1 (PAI-1) is a potent inhibitor of fibrinolysis and in type 2 DM, PAI-1 concentrations are directly associated with hyperinsulinaemia. The net result of these abnormalities of increased coagulation and decreased fibrinolysis is an enhanced tendency for thrombus formation.

Elevated PAI-1 and fibrinogen levels correlate with other features of the metabolic syndrome such as fasting plasma insulin, body mass index, increases in blood pressure and triglycerides. PAI-1 is therefore a strong cardiovascular risk marker in type 2 diabetes and non-diabetic overweight subjects with insulin resistance [42].

Before troglitazone was withdrawn from clinical use, it had been shown to decrease PAI-1 levels [43]. Rosiglitazone, when added to a sulphonylurea treatment in type 2 DM subjects, reduced significantly PAI-1 levels by 22% over a 6 week period compared with the sulphonylurea alone [44]. These results are important because of the increased risk of premature atherosclerotic events in type 2 diabetes.

ENDOTHELIAL DYSFUNCTION,INFLAMMATION AND ATHEROSCLEROSIS

Insulin resistance is associated with endothelial dysfunction, and the latter appears to precede the development of overt type 2 DM. The progression of endothelial dysfunction to atherosclerotic changes in blood vessels seems to parallel the progression of this insulin resistance to type 2 DM [45, 46]. As described earlier, insulin resistance is closely associated with central obesity. This visceral adiposity results in increased circulating free fatty acids which inhibit insulin-mediated glucose uptake. In addition, the fat cells secrete increased amounts of inflammatory adipocytokines, such as tumour necrosis factor α (TNFα) and interleukin-6 (IL-6) which induce insulin resistance, and in contrast the adipocytes secrete a decreased production of insulin-sensitising adipokines, such as adiponectin [47, 48].

Endothelial dysfunction and inflammation are closely interlinked and lead to atherosclerosis. Endothelial damage leads to a reduction in the release of nitric oxide (No) which is a potent vasodilator. There is a balance between No production by endothelial No synthase (eNOS) and degradation by reactive oxygen species (ROS). Increased ROS activity attributes to endothelial dysfunction. The vasculature is therefore less reactive. This endothelial dysfunction also leads to an increase in adhesion molecules, such as VCAM (vascular cell adhesion molecules) and ICAM (intracellular cell adhesion molecules) on the cell surface, attracting increasing numbers of monocytes which can further release chemokines such as MCP-1 (monocyte chemoattractant protein-1), the production of which is stimulated by oxidised LDL-cholesterol. MCP-1 is a crucial factor in the early stages of atherosclerosis. In the vessel wall, cytokines (TNFα and IL-6) are released causing a local inflammatory process with fibrosis (atherosclerotic fibrous cap). Unstable plaque rupture can occur due to release of MMP-9, a matrix metalloproteinase, which breaks down the collagen in the fibrous cap, and is a recognised marker of plaque vulnerability and risk of rupture.

Furthermore, the inflammatory cytokines act on the liver to release acute phase proteins such as C-reactive protein (CRP). Elevated levels of CRP correlate with insulin resistance, are predictive of development of type 2 DM, and also of cardiovascular events. Any rise in CRP is associated with a two to four fold increased risk of vascular events. The 'traditional' markers of the metabolic

syndrome are those described by Reaven in 1988 [4], but those new or emerging 'non traditional' markers, involved in endothelial dysfunction and oxidative stress, vascular inflammation and plaque destabilisation have been extensively reviewed [49]. Their optimal use in routine screening and risk stratification has yet to be determined [50]. Nevertheless, therapies such as TZDs, which improve these 'non traditional' risk factors, are attracting much interest as they may improve the outlook for cardiovascular disease in type 2 DM subjects. The so-called pleiotrophic actions of TZDs may modulate the onset and development of metabolic disorders pre-disposing to atherosclerosis and exert direct anti-atherogenic actions at the level of the vessel wall [51, 52].

Although the results from large prospective studies for both pioglitazone and rosiglitazone are currently awaited to determine whether TZDs will influence macrovascular events in type 2 DM patients, there is now an increasing body of evidence involving these 'non traditional' risk factors to support a beneficial vascular effect of TZDs. The pleiotrophic benefits of TZDs on endothelial dysfunction, inflammation and atherosclerosis are summarised in Table **2**.

Endothelial Dysfunction

Elevated ROS levels which contribute to endothelial dysfunction are reduced by rosiglitazone [53]. Rosiglitazone has also been shown to improve endothelium-dependent flow-mediated dilation [54]. These effects will lead to improved vascular reactivity. Studies with rosiglitazone and pioglitazone in type 2 DM patients have shown that these vascular protective effects are independent of insulin sensitivity [55, 56], which suggests there is a direct action of TZDs on the vasculature [51].

Vascular Inflammation

Both TZDs, pioglitazone and rosiglitazone, have been shown to lower serum levels of inflammatory biomarkers of atherosclerosis. In one study, rosiglitazone therapy for 26 weeks reduced serum levels of the pro-inflammatory marker CRP and MMP-9 and these reductions may indicate beneficial effects on cardiovascular risk [57]. This effect of rosiglitazone has been shown in non-diabetic subjects with atherosclerotic vascular disease as well as in type 2 diabetic patients [58]. In some studies, this anti-inflammatory effect of TZDs could be seen after only two weeks, whereas the blood-glucose lowering effect of TZDs is usually seen after 6-8 weeks, which suggests PPAR γ agonists have these effects independent of their metabolic action [59].

Adiponectin is an adipocytokine which has anti-inflammatory properties, ameliorates insulin resistance and is now considered to have direct anti-atherogenic actions [48]. Treatment with TZDs significantly increases serum adiponectin levels in type 2 DM patients [56, 60, 61]. Increased adiponectin levels are associated with better glycaemic control, a better lipid profile and reduced inflammation in diabetic subjects [62]. In type 2 DM subjects pioglitazone has been shown to increase plasma adiponectin and this is strongly associated with a decrease in hepatic fat content and improvements in hepatic and peripheral insulin sensitivity [63]. Adiponectin may be the unifying link between insulin resistance and inflammation. These effects of TZDs on adiponectin are felt to be an important action of TZD therapy in ameliorating the atherosclerotic process and further studies in this particular area are awaited with interest.

Atherosclerosis

There is experimental evidence that TZDs may reduce MMP-9 and MMP-13, and lessen the chances of plaque rupture and subsequent thrombus formation [49]. As already discussed, TZDs may reduce PAI-1 activity and increase fibrinolytic activity.

These beneficial effects on the atherothrombotic process are now being seen in a more clinical setting with sophisticated radiological techniques showing improvements, and indeed possible reversal of atherosclerosis in coronary and carotid vasculature. Coronary atherosclerosis can be studied with intravascular ultrasound scanning. Pioglitazone has been shown to reduce intimal

Table 2. Pleiotropic Effects Of Thiazolidinediones On Endothelial Dysfunction, Inflammation And Atherosclerosis

Endothelial Dysfunction	Vascular Inflammation	Atherosclerosis
↑ vascular reactivity	↓ CRP	↓ MMP-9 (increased plaque stability)
↓ ROS	↓ MCP-1	↓ MMP-13 (increased plaque stability)
	↓ TNFα	
	↓ IL-6	↓ PAI-1 (increased fibrinolysis)
	↑ Adiponectin	

tissue proliferation after coronary artery stenting [64]. After six months of rosiglitazone therapy, there was a significant reduction in stent restenosis compared with placebo [65], but this benefit was not seen in another study with rosiglitazone [66].

There is now evidence that both TZDs can decrease carotid arterial intima-media thickness (IMT) in type 2 DM subjects [67, 68] which may influence clinical outcome with regard to atherosclerotic ischaemic stroke disease.

OUTCOME TRIALS TO ASSESS REDUCTION OF CARDIOVASCULAR RISK IN TYPE 2 DM PATIENTS

Both pioglitazone and rosiglitazone are undergoing clinical trials to assess their ability in reducing the cardiovascular risk of type 2 DM patients. Table **3** summarises some of the selected on-going trials involving pioglitazone and rosiglitazone. The principal large prospective trial for pioglitazone is the PROactive study and for rosiglitazone, the RECORD study.

The PROactive study will assess the effect of pioglitazone on the secondary prevention of macrovascular events in type 2 diabetes. Over 5,000 patients who have type 2 DM and a history of vascular disease will be randomised to pioglitazone or placebo. The primary end-point is the time from randomisation to the occurrence of a new macrovascular event or death. The study will report in late 2005 [69].

The RECORD study will evaluate the impact of rosiglitazone on the development and progression of cardiovascular disease in type 2 DM subjects. This study will also compare the effects on glycaemic control of sulphonylurea plus metformin combination therapy to rosiglitazone in combination with either a sulphonylurea or metformin. 4,000 patients will be followed up for a period of about 6 years.

These long-term outcome studies with pioglitazone and rosiglitazone will determine if there are beneficial effects of TZDs on vascular end-points to improve the prognosis of type 2 DM patients. From the experimental evidence to hand, the promise for TZDs to influence atherosclerotic vascular disease is compelling but these studies will determine if these benefits on cardiovascular risk factors and atherogenesis itself translate into a reduction in vascular outcomes.

SAFETY PROFILE WITH THIAZOLIDINE-DIONES

Weight gain, usually in the order of 2-5 kg is seen in the first 6 months or so of TZD therapy and as discussed earlier this seems to stabilise as treatment continues. The weight gain is attributed to extra adipose tissue in peripheral subcutaneous sites and not in central visceral fat depots, reflecting the effect of TZDs on new adipocyte formation.

Hepatic impairment was an initial concern for pioglitazone and rosiglitazone when the TZDs were introduced into clinical practice as the original TZD, troglitazone, was withdrawn from clinical use because of fatal cases of liver failure. This has not been seen with either of the TZDs in clinical practice and the idiosyncratic hepatotoxicity seen with troglitazone has been attributed to its Vitamin-E side chain moeity which is not present in the later TZD preparations. However, it is recommended that the serum alanine aminotransferase levels are assessed at the start of TZD therapy and thereafter every two months or so for the first year of treatment.

Fluid retention is a common feature of TZD therapy and is most likely dose dependent. Peripheral oedema occurs in about 5-10% of patients on TZD therapy. In about 2% of patients the TZD may have to be discontinued and in the others the oedema will respond to loop-diuretic therapy.

As type 2 DM patients have cardiac problems, there has been concern that this fluid retention may increase the risk of heart failure [70]. The mechanism of the oedema is not clear [71], but it is particularly seen when TZDs are combined with insulin therapy in type 2 DM patients. The incidence of congestive cardiac failure was similar with pioglitazone (12/1857 cases) and non-pioglitazone treatments (10/1856) in over 3,700 patients assessed over 12 months in type 2 DM patients randomised to either pioglitazone, metformin or gliclazide therapy [72]. Nevertheless at the present state of knowledge it is prudent to be

Table 3. Cardiovascular Trials In Type 2 Diabetes Mellitus Involving Pioglitazone And Rosiglitazone

• ACCORD	Action to Control CardiOvascular Risk in Diabetes (rosiglitazone)
• BARI-2D	Bypass Angioplasty Revascularisation Investigation-type 2 Diabetes (pioglitazone and rosiglitazone)
• CHICAGO	A study evaluating Carotid intima-media tHICkness (CIMT) in Atherosclerosis using pioGlitazOne
• DREAM	Diabetic REduction Approaches with ramipril and rosiglitazone Medications
• PERISCOPE	Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation
• PROACTIVE	PROspective pioglitAzone Clinical Trial In macroVascular Events
• RECORD	Rosiglitazone Evaluated Cardiac Outcomes and Regulation of glycaemia in Diabetes

aware of the risk of congestive cardiac failure when TZDs are used in subjects with type 2 DM until the results of prospective clinical trials are available to further determine the cardiovascular safety of TZDs in diabetic patients with underlying heart disease [73]. Particular care should be taken in patients with uncontrolled diabetes and systolic heart failure, with a low starting dose of TZD and slow and careful titration of the drug escalation over several months.

FUTURE CLINICAL DEVELOPMENTS WITH TZDS

There is growing interest that TZDs may have a protective effect on beta-cell function. In the USA, Diabetes Prevention Program (DPP), although troglitazone therapy was stopped after a mean of 10 months, the conversion rate to type 2 DM remained decreased after 4 years, suggesting a long-lasting effect of TZD treatment on beta-cell function [74]. Further evidence for a protective effect of TZDs on beta-cell function came from the Troglitazone in Prevention of Diabetes (TRIPOD) study. Troglitazone therapy in Hispanic females with a previous history of gestational diabetes decreased the conversion rate of IGT to overt type 2 DM by 55% [75]. After the withdrawal of troglitazone, these results have been reproduced in the pioglitazone extension of the study, PIPOD [76]. Earlier experimental work with troglitazone in diabetic rats demonstrated restoration of insulin secretory capacity with beta-cell regranulation seen on histological examination of the pancreas [77].

How TZDs may give beta-cell protection is unclear but they may do so by reducing lipotoxicity in the beta-cells [78], by preserving apoptosis of beta-cells [79], and there may also be direct effects of TZDs on PPARγ receptors on the beta-cell membrane to preserve beta-cell function [80]. Whatever the exact cause or causes of this possible beta-cell protection, the clinical benefits of such effects would allow continued beta-cell function and prevent the need for type 2 DM patients to go onto combinations of oral agent therapy or eventually to insulin treatment. The earlier that such TZD therapy is started, the greater the potential benefit. Such an early intervention study is underway in the USA with pioglitazone ('Actos'), entitled ACTos NOW ("ACT NOW") to compare pioglitazone versus placebo over a 39 month period in conversion of IGT to type 2 DM. This study may determine whether TZD treatment has an established place in the management of IGT.

THIAZOLIDINEDIONE USE IN OTHER CLINICAL CONDITIONS

This review of PPARγ agonism has concentrated on the role of TZDs in the management of insulin resistance and its consequences as seen in type 2 DM and its associated clinical sequelae, the so-called 'silent sextet'. Indeed type 2 DM is currently the only approved indication for TZD treatment. However, there is on-going clinical research into the use of TZDs and other medical conditions associated with insulin resistance such as polycystic ovarian syndrome, lipodystrophies such as that seen with human immunodeficiency viral (HIV) disease, and non alcohol steatotic hepatitis (NASH), which have been recently reviewed [39].

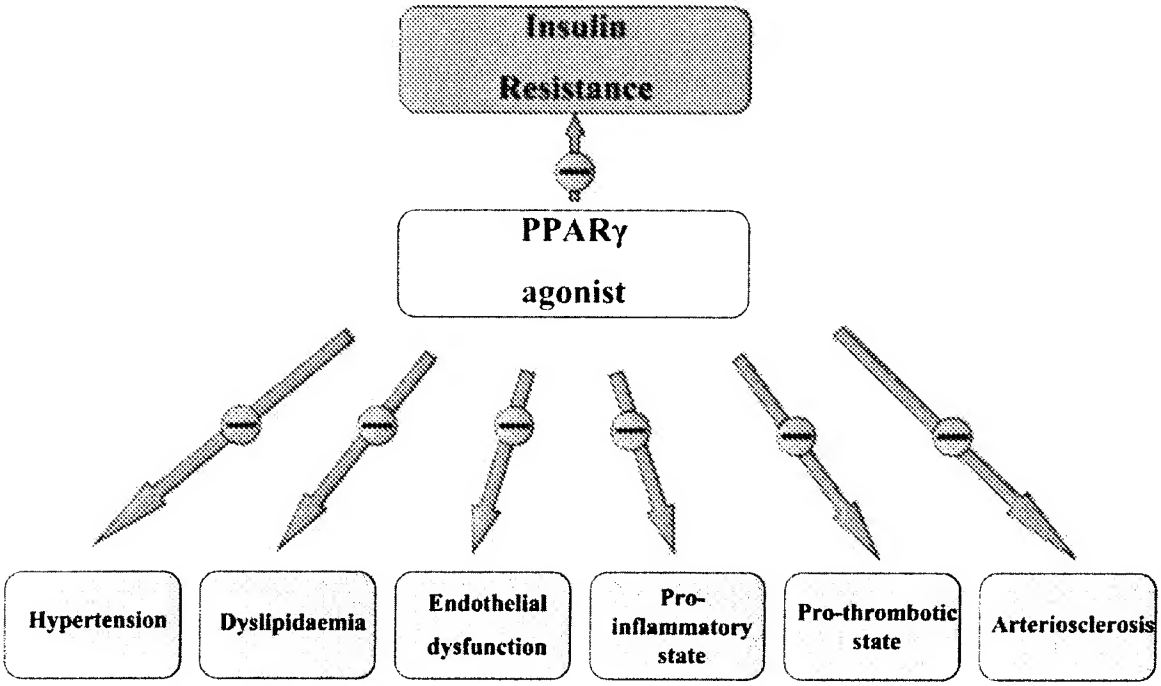


Fig. (10). PPAR γ agonists (TZDs) directly influence insulin resistance and modulate cardiovascular risk factors associated with atherosclerosis. Adapted from ref 59, with permission.

CONCLUSIONS

Type 2 DM has now reached epidemic proportions world-wide. TZDs represent a new class of oral hypoglycaemic agents which not only tackle the core problem of insulin resistance itself but also modulate the associated risk factors and development of atherosclerosis seen in cardiovascular disease Fig. **(10)** [59,81]. The TZDs also have the possibility of preserving beta-cell function [82]. Despite their benefits on insulin resistance, the weight of type 2 DM patients may rise and anti-hypertensive drugs, statins and fibrates along with other lipid lowering agents will still be required to treat both the hypertension and dyslipidaemia seen so frequently in type 2 DM subjects. Nevertheless, TZDs have the potential to directly influence atherogenesis and the results of prospective trials with both pioglitazone and rosiglitazone to see how these agents may reduce cardiovascular events in type 2 DM subjects are eagerly awaited. The experimental work and early clinical studies hold out this promise of vascular protection – will it be fulfilled?

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